# Involvement of NMDA glutamate receptors in the acquisition and reinstatement of the conditioned place preference induced by MDMA

Maria P. García-Pardo, Carla Escobar-Valero, Marta Rodríguez-Arias,
Jose Miñarro and Maria A. Aguilar

**Some 3,4-methylenedioxymethamphetamine (MDMA) users become dependent as a result of chronic consumption. A greater understanding of the neurobiological basis of the rewarding effects of MDMA could contribute to developing effective pharmacotherapies for MDMA-related problems. The present study evaluated the role of *N*-methyl-D-aspartate (NMDA) glutamate receptors (NMDARs) in the acquisition and reinstatement of conditioned place preference (CPP) induced by MDMA. Adolescent male mice were conditioned with 1 or 10 mg/kg MDMA and pretreated with 5 or 10 mg/kg of the NMDAR antagonist memantine during acquisition of conditioning (experiment 1), or before a reinstatement test (experiment 2). In addition, the effects of memantine on acquisition of chocolate-induced CPP and the effects of memantine and MDMA on a passive avoidance task were evaluated. Memantine did not exert any motivational effects, but blocked the acquisition of MDMA-induced CPP. Moreover, following acquisition and extinction of MDMA-induced CPP, memantine did not induce reinstatement but blocked reinstatement of the CPP induced by priming with MDMA. Memantine did not block the CPP induced by chocolate, and it partially reversed the impairing effects of MDMA on memory. Our results demonstrate that NMDARs are involved in acquisition of the conditioned rewarding effects of MDMA and in priming-induced reinstatement of CPP following extinction. Moreover, they suggest the validity of memantine for the treatment of MDMA abuse. *Behavioural Pharmacology* 26:411–417 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.**

**Behavioural Pharmacology** 2015, **26:**411–417

**Keywords: adolescence, conditioned place preference, memantine, 3,4-methylenedioxymethamphetamine, mice, *N*-methyl-D-aspartate glutamate receptors**

Unit of Research Psychobiology of Drug Dependence, Department of Psychobiology, Faculty of Psychology, University of Valencia, Spain

Correspondence to Maria A. Aguilar, PhD, Departamento de Psicobiología, Facultad de Psicología, Universitat de Valencia, Avda. Blasco Ibáñez, 21, 46010 Valencia, Spain
E-mail: asuncion.aguilar@uv.es

**Received** 8 August 2014 **Accepted as revised** 13 March 2015

## Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a popular amphetamine derivative consumed largely by adolescents and young adults in the context of weekend nightlife (EMCDDA, 2013). Although its abuse potential is not as high as that of some other compounds, MDMA dependence has been documented as a result of chronic consumption (Cottler *et al.*, 2001; Degenhardt *et al.*, 2010; Leung *et al.*, 2010). Animal models of reward, such as self-administration (SA) and conditioned place preference (CPP), also point to MDMA as a drug of abuse that induces reinstatement after extinction (Schenk, 2009; Schenk *et al.*, 2011; Roger-Sánchez *et al.*, 2013a), an animal model of relapse to drug seeking after long periods of abstinence (Aguilar *et al.*, 2009a; Bossert *et al.*, 2013). Several neurotransmitter systems have been implicated in the acquisition and/or reinstatement of the rewarding effects of MDMA in the SA and CPP paradigms: serotonin [5-hydroxytryptamine (5-HT)], dopamine (DA), endogenous opioids, endocannabinoids, neurotensin and adenosine (for a review, see Roger-Sánchez *et al.*, 2013a).

Although the glutamatergic system is clearly involved in drug addiction (Olive *et al.*, 2012; Pomierny-Chamiolo *et al.*, 2013; Tomek *et al.*, 2013), and blockade of *N*-methyl-D-aspartate (NMDA) glutamate receptors (NMDARs) decreases the rewarding effect of several drugs of abuse (Ribeiro Do Couto *et al.*, 2005; Popik *et al.*, 2006; Maldonado *et al.*, 2007), the involvement of these receptors in MDMA reward has not been studied. Conversely, previous studies have demonstrated that NMDARs are implicated in other effects of MDMA. For example, memantine and other NMDAR antagonists decrease MDMA-induced hyperthermia (Johnson *et al.*, 2004; Nisijima *et al.*, 2012) and prevent MDMA-induced neurotoxicity (Miller and O'Callaghan, 1995; Finnegan and Taraska, 1996; Johnson *et al.*, 2004; Chipana *et al.*, 2008), although, in the former case, this effect could be attributed to their action as an α-7 nicotinic cholinergic receptors antagonist (Chipana *et al.*, 2008). Moreover, MDMA administration has been shown to enhance the extracellular concentration of glutamate in the rat hippocampus (Anneken and Gudelsky, 2012). In light of this evidence, we hypothesized that NMDARs are also

0955-8810 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved. DOI: 10.1097/FBP.0000000000000138

involved in MDMA reward. Thus, the aim of the present study was to evaluate the effects of the noncompetitive NMDAR antagonist memantine on acquisition (experiment 1) and reinstatement (experiment 2) of an MDMA-induced CPP. In addition, as part of experiment 1, we also tested the effects of memantine on acquisition of chocolate-induced CPP to demonstrate that this drug did not impair contextual learning of another appetitive substance. Finally, we evaluated the consequences of memantine administration on the impairing effects of MDMA on passive avoidance learning to determine whether the NMDAR antagonist affected learning of a different conditioning task (experiment 3). We used adolescent mice in these experiments, as MDMA is a drug predominately consumed by adolescents and young adults.

## Methods

### Subjects and treatments

A total of 272 OF1 male mice (Charles River, Barcelona, Spain) that were 21 days old and group-housed under standard laboratory conditions were used (see details in Roger-Sánchez *et al.*, 2013a). Procedures involving mice and their care were conducted in compliance with the Directive 2010/63/EU. Animals were injected intraperitoneally with MDMA hydrochloride (Agencia Española del Medicamento, Madrid, Spain) and/or memantine (Tocris Bioscience Laboratories, Bristol, UK) in a volume of 0.01 ml/g. Control groups were injected with the physiological saline in which the drugs were dissolved (0.9% NaCl). The doses were selected on the basis of previous studies on MDMA (Daza-Losada *et al.*, 2007, 2009a; Manzanedo *et al.*, 2010; Rodríguez-Arias *et al.*, 2010; Vidal-Infer *et al.*, 2012) and memantine (Ribeiro Do Couto *et al.*, 2004; Ribeiro Do Couto *et al.*, 2005; Maldonado *et al.*, 2007).

### Place conditioning

For place conditioning, we used 12 computerized Plexiglas boxes, with two equally sized compartments separated by a grey central area (see details in Roger-Sánchez *et al.*, 2013a). The compartments had different coloured walls (black vs. white) and distinct floor textures (fine grid in the black compartment and wide grid in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the position of the animals and their crossings from one compartment to the other to be recorded. The equipment was controlled by three IBM PC computers using MONPRE 2Z software (CIBERTEC, SA, Spain).

The CPP test began on postnatal day (PND) 29, following an unbiased procedure (in terms of initial preference and counterbalanced drug treatment in both compartments) with three phases (for details, see Daza-Losada *et al.*, 2007): preconditioning (Pre-C), conditioning (drug on PND 32, 34, 36 and 38 and saline on alternate days, confinement for 30 min) and post-conditioning (Post-C). We assessed the time spent by the mice in each compartment in the Pre-C and Post-C tests during a 15 min period. A significant increase in the time spent in the drug-paired compartment during Post-C compared with Pre-C indicated the acquisition of CPP.

To study the implication of NMDAR in the acquisition of MDMA-induced CPP (experiment 1), six groups of early-adolescent animals were conditioned (PND 32–39) with physiological saline (Sal + Sal, $n = 10$), 5 or 10 mg/kg memantine (M5 + Sal, $n = 10$; M10 + Sal, $n = 12$), 10 mg/kg MDMA (Sal + MDMA10, $n = 15$), or 5 or 10 mg/kg memantine plus 10 mg/kg MDMA (M5 + MDMA10, $n = 12$; M10 + MDMA10, $n = 13$). An additional three groups of mice ($n = 10$) were used to demonstrate the effects of memantine on CPP induced by an additional dose of MDMA in late-adolescent animals. In this case, mice were conditioned (PND 53–60) with 1 mg/kg MDMA plus saline (Sal + MDMA1) or 5 or 10 mg/kg memantine (M5 + MDMA1, M10 + MDMA1). Memantine or saline was administered 30 min before MDMA administration, and the animals underwent conditioning immediately after the second injection. The Post-C test was performed on PND 40 and PND 61 in early-adolescent and late-adolescent mice, respectively.

To demonstrate that memantine did not impair contextual learning of another appetitive substance, three additional groups of mice were conditioned (PND 32–39) with chocolate (ChocoKrispies; Kelloggs, Tarragona, España) following the same procedure (CHOC + Sal, $n = 11$; CHOC + M5, $n = 12$; CHOC + M10, $n = 11$). To ensure acquisition of CPP, the mice were familiarized with the chocolate in their cage over 6 days, removing it 3 days before initiation of conditioning. The animals were deprived of food for 8 h before each conditioning session with chocolate.

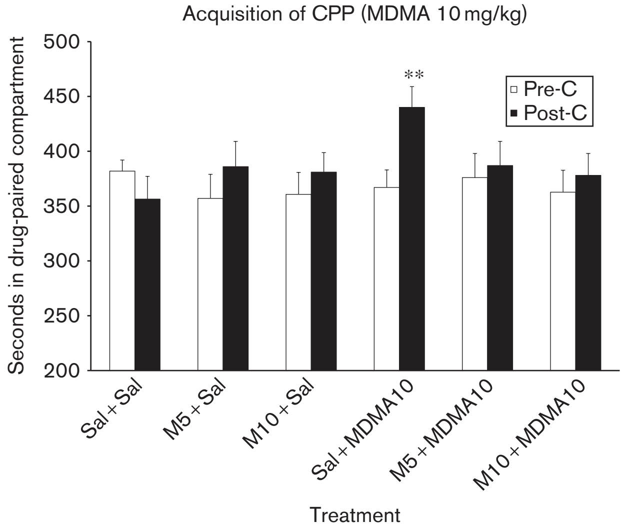
To study the implication of NMDAR in the reinstatement of MDMA-induced CPP (experiment 2), six groups of animals were conditioned with 10 mg/kg MDMA (PND 32–39) and, after Post-C, were subjected to an extinction/reinstatement procedure (for details, see Roger-Sánchez *et al.*, 2013a). The mice were subjected to an extinction procedure every 3 days (as described previously by García-Pardo *et al.*, 2014) until they achieved complete extinction of CPP (between three and six sessions among the different groups). CPP was considered to be extinguished when there was no significant difference between the time spent in the drug-paired compartment in the extinction session and that spent in the same compartment during Pre-C (Student's *t*-test).

On the following day, the animals performed the reinstatement test after administration of the following: physiological saline (Sal + Sal, $n = 12$), 5 mg/kg MDMA (Sal + MDMA5, $n = 11$), 5 or 10 mg/kg memantine (M5 + Sal, $n = 10$; M10 + Sal, $n = 11$), or 5 or 10 mg/kg memantine + 5 mg/kg MDMA (M5 + MDMA5, *n* = 10; M10 + MDMA5, *n* = 10). Memantine or saline was administered 15 min before MDMA or saline, and the animals were tested 15 min after the second injection. It is important to note that the interval between injections differed from that in experiment 1 (15 vs. 30 min, respectively); in this experiment, the mice were placed in the CPP apparatus 15 min after rather than immediately after the second injection (experiment 1). Thus, in both cases, memantine was administered 30 min before the mice were placed in the CPP apparatus.

### Passive avoidance

In experiment 3, four groups of mice were treated on four alternate days with saline (Sal + Sal, *n* = 8), 10 mg/kg MDMA (Sal + MDMA10, *n* = 13), or 10 mg/kg MDMA plus 5 or 10 mg/kg memantine (M5 + MDMA10, *n* = 14; M10 + MDMA10, *n* = 14) to determine whether memantine affected the detrimental effects of MDMA on the passive avoidance test. Memantine was administered 30 min before MDMA. Mice were trained 4 days after treatment in a step-through inhibitory avoidance apparatus (Ugo Basile, Comerio-Varese, Italy). Details of the apparatus and the procedure have been described elsewhere (Daza-Losada *et al.*, 2009b). In brief, on the training day, each mouse was placed in the illuminated compartment of the apparatus and received a footshock (0.5 mA, 3 s) when it entered the dark compartment, after which it was returned to its home cage. The time taken to enter the dark compartment (step-through latency) was recorded. Retention was tested 24 h and 1 week later, following the same procedure but without the shock and with a maximum step-through latency of 300 s.

**Fig. 1**



Effects of memantine on the acquisition of place conditioning induced by 10 mg/kg MDMA. During the conditioning phase, animals were divided into the following six treatment groups: saline (Sal + Sal, *n* = 10), 5 or 10 mg/kg memantine (M5 + Sal, *n* = 10; M10 + Sal, *n* = 12), saline + 10 mg/kg MDMA (Sal + MDMA10, *n* = 15) and 5 or 10 mg/kg memantine + 10 mg/kg MDMA (M5 + MDMA10, *n* = 12; M10 + MDMA10, *n* = 13). The bars represent the mean (± SEM) time (s) spent in the drug-paired compartment before conditioning sessions in the preconditioning test (Pre-C, white bars) and after conditioning sessions in the postconditioning test (Post-C, black bars). $^{**}P < 0.001$, Pre-C versus Post-C tests. CPP, conditioned place preference; M, memantine; MDMA, 3,4-methylenedioxymethamphetamine; Sal, saline.

### Statistical analysis

The time spent in the drug-paired compartment by early-adolescent mice was analysed with a mixed analysis of variance (ANOVA), with two between-subject variables (MDMA and memantine) and one within-subject variable (days). Data obtained for mice conditioned with 1 mg/kg MDMA or chocolate and for passive avoidance were analysed using a two-way ANOVA, with one between-subject variable (treatment) and one within-subject variable (days). Bonferroni adjustment was used to make post-hoc comparisons.

## Results

### Experiment 1: involvement of NMDAR in the acquisition of the CPP induced by MDMA

For animals receiving 10 mg/kg MDMA, there was a significant effect of 'days' [$F(1,66) = 6.12$, $P < 0.05$] and a significant 'days × MDMA × memantine' interaction [$F(2,66) = 5.02$, $P < 0.01$]. Only the group treated with Sal + MDMA spent more time in the drug-paired compartment in Post-C than in Pre-C ($P < 0.001$). This group also spent more time than the Sal + Sal group in the drug-paired compartment during Post-C ($P < 0.01$). The absence of CPP in the groups receiving memantine suggests that this drug prevented the acquisition of MDMA-induced CPP (Fig. 1).

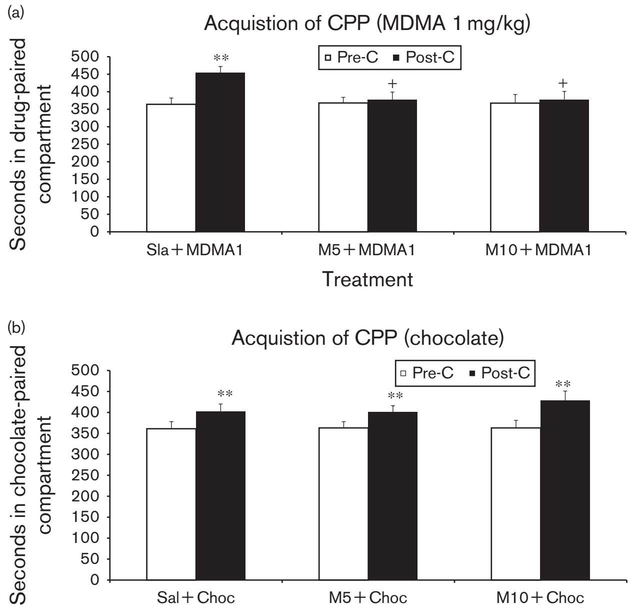
Similarly, the ANOVA for mice conditioned with 1 mg/kg MDMA also showed a significant effect of 'days' [$F(1,27) = 7.27$, $P < 0.05$] and a significant 'days × treatment' interaction [$F(2,27) = 3.89$, $P < 0.05$]. Mice conditioned with MDMA alone spent more time in the drug-paired compartment in the Post-C test than in the Pre-C test ($P < 0.001$), and compared with the groups treated with MDMA plus memantine ($P$'s $< 0.05$). Again, the absence of CPP in the groups receiving memantine suggests that this drug prevented acquisition of MDMA-induced CPP (Fig. 2a).

The ANOVA of mice conditioned with chocolate showed that only the effect of 'days' was significant [$F(1,31) = 32.98$; $P < 0.001$], with all groups spending more time in the drug-paired compartment during Post-C than during Pre-C. This showed that memantine did not block the CPP induced by chocolate (Fig. 2b).

### Experiment 2: involvement of NMDAR in the reinstatement of MDMA-induced CPP

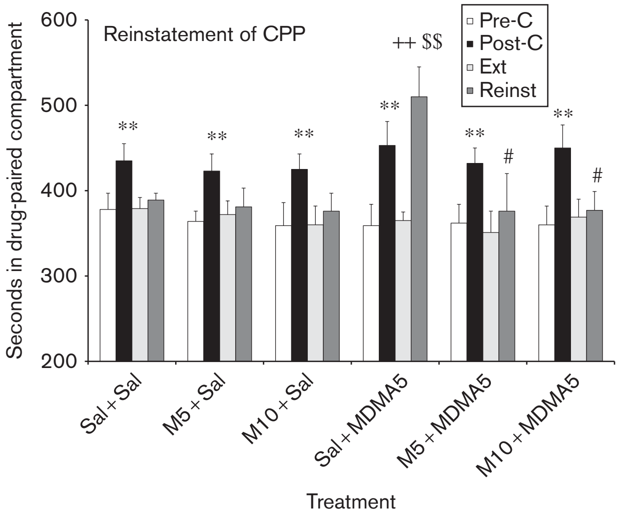
In this experiment, only the variable 'days' was found to be significant [$F(3,56) = 14.26$, $P < 0.001$]. The mice

**Fig. 2**



(a) Effects of memantine on acquisition of the place conditioning induced by 1 mg/kg MDMA. During the conditioning phase, animals were divided into the following three treatment groups: saline + 1 mg/kg MDMA (Sal + MDMA1, *n* = 10), and 5 or 10 mg/kg memantine + 1 mg/kg MDMA (M5 + MDMA1, *n* = 10; M10 + MDMA1, *n* = 10). (b) Effects of memantine on acquisition of the place conditioning induced by chocolate. During the conditioning phase, animals were divided into the following three treatment groups: saline + chocolate (Sal + Choc, *n* = 11), and 5 or 10 mg/kg memantine + chocolate (M5 + Choc, *n* = 12; M10 + Choc, *n* = 11). The bars represent the mean (± SEM) time (s) spent in the drug-paired compartment before conditioning sessions in the preconditioning test (Pre-C, white bars) and after conditioning sessions in the postconditioning test (Post-C, black bars). $^{**}P<0.01$, Pre-C versus Post-C tests. $^{+}P<0.05$, with respect to the Sal + MDMA1 group. Choc, chocolate; CPP, conditioned place preference; M, memantine; MDMA, 3,4-methylenedioxymethamphetamine; Sal, saline.

**Fig. 3**



Effects of memantine on the reinstatement of MDMA-induced CPP. Six groups of mice were conditioned with 10 mg/kg MDMA, underwent daily extinction sessions until the CPP was extinguished, and received the following treatments before the reinstatement test: two injections of saline (Sal + Sal, *n* = 12), saline + 5 mg/kg MDMA (Sal + MDMA5, *n* = 11), 5 or 10 mg/kg memantine (M5 + Sal, *n* = 10; M10 + Sal; *n* = 11), and 5 or 10 mg/kg memantine + 5 mg/kg MDMA (M5 + MDMA5, *n* = 10; M10 + MDMA5, *n* = 10). The bars represent the mean (± SEM) time (s) spent in the drug-paired compartment before conditioning sessions in the preconditioning (Pre-C) test (white bars), after conditioning sessions in the postconditioning (Post-C) test (black bars), in the last extinction session (light grey bars) and in the reinstatement test (dark grey bars). $^{**}P<0.001$, Post-C versus Pre-C or the last extinction tests. $^{++}P<0.001$, reinstatement versus last extinction tests. $^{\$\$}P<0.01$, reinstatement test with respect to the Sal + Sal group. $^{\#}P<0.05$, reinstatement test with respect to the Sal + MDMA5 group. CPP, conditioned place preference; Ext, extinction; M, memantine; MDMA, 3,4-methylenedioxymethamphetamine; Reinst, reinstatement; Sal, saline.

spent more time in the drug-paired compartment during Post-C than during Pre-C ($P<0.001$) and extinction ($P<0.001$). The difference between the time spent in extinction and reinstatement approached significance ($P<0.065$), as only the Sal + MDMA5 group spent more time in the drug-paired compartment during the reinstatement test compared with the last extinction session ($P<0.001$). Moreover, on the reinstatement day, the group receiving Sal + MDMA5 spent more time in the drug-paired compartment compared with the Sal + Sal group ($P<0.01$) and the M5 + MDMA5 and M10 + MDMA5 groups ($P<0.05$). These results indicate that memantine blocked the reinstatement of CPP (Fig. 3).

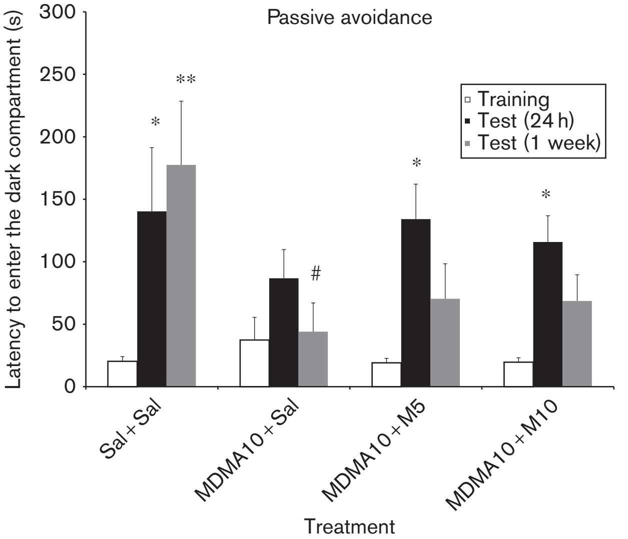
### Experiment 3: involvement of NMDAR in the detrimental effects of MDMA in the passive avoidance test

The ANOVA for this experiment showed a significant effect of 'days' [$F(2,44)=16.61$, $P<0.001$] and a significant 'days × treatment' interaction [$F(6,90)=2.12$, $P<0.05$]. In all groups, except that treated with MDMA alone, the latency to enter the dark compartment increased between day 1 and 2 ($P$s $<0.01$). However, on day 3, only the control group showed an increased latency in comparison with day 1 ($P<0.001$), which was significantly higher than the latency of the group treated with MDMA alone ($P<0.05$). These results suggest that memantine reverses the impairing effects of MDMA on passive avoidance only at an initial stage (Fig. 4).

## Discussion

In the present study we demonstrate, for the first time, that the conditioned rewarding effects of MDMA in mice depend on the activation of glutamate NMDARs. Memantine inhibited acquisition of MDMA CPP and blocked priming-induced reinstatement. These results are consistent with the involvement of the glutamatergic system in learning and memory (Nicoll and Malenka, 1999; Tang *et al.*, 1999; Riedel *et al.*, 2003) and in drug addiction (Olive *et al.*, 2012; Pomierny-Chamioło *et al.*, 2013; Tomek *et al.*, 2013).

**Fig. 4**



Effects of memantine on the impairing effect of MDMA on the passive avoidance task. Four groups of mice were treated with a daily administration (over four alternate days) of saline (Sal + Sal), 10 mg/kg MDMA alone (MDMA10 + Sal), or 10 mg/kg MDMA plus 5 or 10 mg/kg memantine (MDMA10 + M5, MDMA10 + M10). Four days later, all the mice were trained in acquisition of the passive avoidance task. Retention tests were performed 24 h and a week after training. The bars represent the mean (± SEM) latency (in s) to enter into the dark compartment. $^{**}P < 0.001$, $^{*}P < 0.01$, with respect to the training day; $^{\#}P < 0.05$, with respect to the control group on the same day. M, memantine; MDMA, 3,4-methylenedioxymethamphetamine; Sal, saline.

In accordance with our previous studies, MDMA induced CPP and reinstatement after extinction (Daza-Losada *et al.*, 2007; Rodríguez-Arias *et al.*, 2010; Vidal-Infer *et al.*, 2012; Roger-Sánchez *et al.*, 2013a, 2013b, 2013c), indicating that this drug produces long-term alterations that increase the vulnerability of subjects to relapse after abstinence, as occurs with other drugs of abuse. Conversely, memantine did not induce motivational effects, which is also in line with previous studies (Maldonado *et al.*, 2003, 2007; Ribeiro Do Couto *et al.*, 2004, 2005; Olive *et al.*, 2012).

The acquisition and reinstatement of MDMA CPP were blocked by antagonism of NMDAR. There are no previous studies on the involvement of these receptors in the rewarding effects of MDMA, but several studies have showed that memantine blocks the acquisition of CPP induced by morphine (Popik and Danysz, 1997; Popik *et al.*, 2003, 2006; Ribeiro Do Couto *et al.*, 2004, 2005; Aguilar *et al.*, 2009b), cocaine (Kotlinska and Biala, 2000; Maldonado *et al.*, 2007) and ethanol (Gremel and Cunningham, 2009), as well as the reinstatement of CPP induced by morphine (Ribeiro Do Couto *et al.*, 2005; Popik *et al.*, 2006) and cocaine (Maldonado *et al.*, 2007). In the present study, the effects of memantine on acquisition of CPP induced by MDMA were potent and specific. Memantine blocked the acquisition of CPP induced by two different doses of MDMA in early-adolescent and late-adolescent mice but did not affect the CPP induced by chocolate, which is in accordance with previous studies (Popik *et al.*, 2003).

One possible explanation for the effects observed on CPP is that memantine provokes neurochemical changes that undermine the rewarding effects of MDMA. As these effects seem to depend on the activation of DA and 5-HT receptors (Schenk *et al.*, 2011; Vidal-Infer *et al.*, 2012; Roger-Sánchez *et al.*, 2013b, 2013c), it is possible that memantine decreases the levels of these neurotransmitters. Activation of NMDARs stimulates DA release in rat striatal slices, and memantine inhibits this effect (Nankai *et al.*, 1995). Moreover, acute memantine administration reduces DA in the hippocampus and 5-HT in the hippocampus and cortex, increasing the levels of DA in the cortex (Shearman *et al.*, 2006). Thus, the blockade of NMDARs in DA neurons of the ventral tegmental area could counteract the enhancement of DA release in the nucleus accumbens induced by MDMA and, in turn, undermine its rewarding effects. Furthermore, the inhibition of serotonin release induced by memantine and its action as a 5-HT3 antagonist (Rammes *et al.*, 2001) could contribute to the inhibition of the rewarding properties of MDMA. As glutamate is clearly involved in learning and memory (Riedel *et al.*, 2003), it is also possible that memantine interferes with the learning of the classic conditioning needed for the acquisition of CPP and with the capacity of MDMA priming to remind the animal of the association previously learned during conditioning. However, this hypothesis is not supported by the results we obtained for chocolate-induced CPP (which was not affected by memantine) and for the passive avoidance task, where memantine only partially reversed the impairing effects of MDMA on memory. Mice treated with MDMA 4 days before training in the passive avoidance task showed a deficit in memory 24 h and a week after training, in agreement with a previous study from our laboratory (Daza-Losada *et al.*, 2009b). However, memantine only significantly reversed this impairing effect of MDMA in the test performed 24 h after training.

There are two limitations to the present study. The first is the use of mice of different ages (early-adolescent and late-adolescent mice) to test the effects of memantine on the CPP induced by two different doses of MDMA. However, it is important to note that in a previous study we observed that 1 and 10 mg/kg MDMA induced the same CPP in late-adolescent mice (García-Pardo *et al.*, 2014). Thus, the use of mice of different ages allows us to confirm that NMDAR antagonism blocks the CPP induced by two different doses of MDMA in both early-adolescent and late-adolescent mice. The other limitation is the high variability observed in all groups in the passive avoidance test, which makes it difficult to draw unequivocal conclusions from this experiment. However, the results suggest that the impairment in learning induced by MDMA is, at least at short-term, reversed by memantine, in agreement with previous studies (Camarasa *et al.*, 2008).

### Conclusion

The present study demonstrates the involvement of NMDARs in the rewarding effects of MDMA and suggests that NMDAR antagonism can be a useful therapeutic target for the treatment of MDMA-related problems. Future studies will need to evaluate the role of other glutamate receptors.

## Acknowledgements

The authors thank Brian Normanly for his editing of the manuscript. The author(s) are entirely responsible for the scientific content of the paper.

This work was supported by the following grants: Ministerio de Economía y Competitividad, Dirección General de Investigación, PSI2011-24762, Instituto de Salud Carlos III, Red de Trastornos Adictivos (RTA) RD12/0028/0005 and Fondos Feder.

Ministerio de Sanidad, Servicios Sociales e Igualdad. Delegación del Gobierno para el Plan Nacional Sobre Drogas, Proyectos de Investigación sobre Drogodependencias, 2014I007. Generalitat Valenciana, Conselleria de Educación, PROMETEOII/2014/063, Val + id (for MP G-P), Spain.

### Conflicts of interest

There are no conflicts of interest.

## References

Aguilar MA, Rodríguez-Arias M, Miñarro J (2009a). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev* **59**:253–277.

Aguilar MA, Manzanedo C, Do Couto BR, Rodríguez-Arias M, Miñarro J (2009b). Memantine blocks sensitization to the rewarding effects of morphine. *Brain Res* **1288**:95–104.

Anneken JH, Gudelsky GA (2012). MDMA produces a delayed and sustained increase in the extracellular concentration of glutamate in the rat hippocampus. *Neuropharmacology* **63**:1022–1027.

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology (Berl)* **229**:453–476.

Camarasa J, Marimón JM, Rodrigo T, Escubedo E, Pubill D (2008). Memantine prevents the cognitive impairment induced by 3,4-methylenedioxymethamphetamine in rats. *Eur J Pharmacol* **589**:132–139.

Chipana C, Camarasa J, Pubill D, Escubedo E (2008). Memantine prevents MDMA-induced neurotoxicity. *Neurotoxicology* **29**:179–183.

Cottler LB, Womack SB, Compton WM, Ben-Abdallah A (2001). Ecstasy abuse and dependence among adolescents and young adults: applicability and reliability of DSM-IV criteria. *Hum Psychopharmacol* **16**:599–606.

Daza-Losada M, Ribeiro Do Couto B, Manzanedo C, Aguilar MA, Rodríguez-Arias M, Miñarro J (2007). Rewarding effects and reinstatement of MDMA-induced CPP in adolescent mice. *Neuropsychopharmacology* **32**: 1750–1759.

Daza-Losada M, Rodríguez-Arias M, Aguilar MA, Miñarro J (2009a). Acquisition and reinstatement of MDMA-induced conditioned place preference in mice pre-treated with MDMA or cocaine during adolescence. *Addict Biol* **14**:447–456.

Daza-Losada M, Rodríguez-Arias M, Maldonado C, Aguilar MA, Guerri C, Miñarro J (2009b). Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. *Neurotoxicol Teratol* **31**:49–59.

Degenhardt L, Bruno R, Topp L (2010). Is ecstasy a drug of dependence? *Drug Alcohol Depend* **107**:1–10.

EMCDDA (2013). European Drug Report. Available at: *http://www.emcdda.europa.eu/edr2013* [Accessed 6 June 2014].

Finnegan KT, Taraska T (1996). Effects of glutamate antagonists on methamphetamine and 3,4-methylenedioxymethamphetamine-induced striatal dopamine release in vivo. *J Neurochem* **66**:1949–1958.

García-Pardo MP, Rodríguez-Arias M, Maldonado C, Manzanedo C, Miñarro J, Aguilar MA (2014). Effects of acute social stress on the conditioned place preference induced by MDMA in adolescent and adult mice. *Behav Pharmacol* **25**:532–546.

Gremel CM, Cunningham CL (2009). Involvement of amygdala dopamine and nucleus accumbens NMDA receptors in ethanol-seeking behavior in mice. *Neuropsychopharmacology* **34**:1443–1453.

Johnson EA, O'Callaghan JP, Miller DB (2004). Brain concentrations of d-MDMA are increased after stress. *Psychopharmacology (Berl)* **173**: 278–286.

Kotlinska J, Biala G (2000). Memantine and ACPC affect conditioned place preference induced by cocaine in rats. *Pol J Pharmacol* **52**:179–185.

Leung KS, Ben Abdallah A, Copeland J, Cottler LB (2010). Modifiable risk factors of ecstasy use: risk perception, current dependence, perceived control, and depression. *Addict Behav* **35**:201–208.

Maldonado C, Cauli O, Rodríguez-Arias M, Aguilar MA, Miñarro J (2003). Memantine presents different effects from MK-801 in motivational and physical signs of morphine withdrawal. *Behav Brain Res* **144**:25–35.

Maldonado C, Rodríguez-Arias M, Castillo A, Aguilar MA, Miñarro J (2007). Effect of memantine and CNQX in the acquisition, expression and reinstatement of cocaine-induced conditioned place preference. *Prog Neuropsychopharmacol Biol Psychiatry* **31**:932–939.

Manzanedo C, Rodríguez-Arias M, Daza-Losada M, Maldonado C, Aguilar MA, Miñarro J (2010). Effect of the CB1 cannabinoid agonist WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference in mice. *Behav Brain Funct* **6**:19.

Miller DB, O'Callaghan JP (1995). The role of temperature, stress, and other factors in the neurotoxicity of the substituted amphetamines 3,4-methylenedioxymethamphetamine and fenfluramine. *Mol Neurobiol* **11**:177–192.

Nankai M, Fage D, Carter C (1995). Striatal NMDA receptor subtypes: the pharmacology of *N*-methyl-D-aspartate-evoked dopamine, gamma-aminobutyric acid, acetylcholine and spermidine release. *Eur J Pharmacol* **286**:61–70.

Nicoll RA, Malenka RC (1999). Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. *Ann N Y Acad Sci* **868**: 515–525.

Nisijima K, Kuboshima K, Shioda K, Yoshino T, Iwamura T, Kato S (2012). Memantine attenuates 3,4-methylenedioxymethamphetamine-induced hyperthermia in rats. *Neurosci Lett* **531**:198–203.

Olive MF, Cleva RM, Kalivas PW, Malcolm RJ (2012). Glutamatergic medications for the treatment of drug and behavioral addictions. *Pharmacol Biochem Behav* **100**:801–810.

Pomierny-Chamioło L, Rup K, Pomierny B, Niedzielska E, Kalivas PW, Filip M (2013). Metabotropic glutamatergic receptors and their ligands in drug addiction. *Pharmacol Ther* **142**:281–305.

Popik P, Danysz W (1997). Inhibition of reinforcing effects of morphine and motivational aspects of naloxone-precipitated opioid withdrawal by *N*-methyl-D-aspartate receptor antagonist, memantine. *J Pharmacol Exp Ther* **280**:854–865.

Popik P, Wrobel M, Rygula R, Bisaga A, Bespalov AY (2003). Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. *Behav Pharmacol* **14**:237–244.

Popik P, Wrobel M, Bisaga A (2006). Reinstatement of morphine-conditioned reward is blocked by memantine. *Neuropsychopharmacology* **31**:160–170.

Rammes G, Rupprecht R, Ferrari U, Zieglgänsberger W, Parsons CG (2001). The *N*-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT(3) receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner. *Neurosci Lett* **306**:81–84.

Ribeiro Do Couto B, Aguilar MA, Manzanedo C, Rodríguez-Arias M, Miñarro J (2004). Effects of NMDA receptor antagonists (MK-801 and memantine) on the acquisition of morphine-induced conditioned place preference in mice. *Prog Neuropsychopharmacol Biol Psychiatry* **28**:1035–1043.

Ribeiro Do Couto B, Aguilar MA, Manzanedo C, Rodríguez-Arias M, Miñarro J (2005). NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. *Brain Res Bull* **64**:493–503.

Riedel G, Bettina P, Jacques M (2003). Glutamate receptor function in learning and memory. *Behav Brain Res* **140**:1–47.

Rodríguez-Arias M, Manzanedo C, Roger-Sánchez C, Do Couto BR, Aguilar MA, Miñarro J (2010). Effect of adolescent exposure to WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference. *Prog Neuropsychopharmacol Biol Psychiatry* **34**:166–171.

Roger-Sánchez C, Aguilar MA, Manzanedo C, Miñarro J, Rodríguez-Arias M (2013a). Neurochemical substrates of MDMA reward: effects of the inhibition of serotonin reuptake on the acquisition and reinstatement of MDMA-induced CPP. *Curr Pharm Des* **19**:7050–7064.

Roger-Sánchez C, Rodríguez-Arias M, Miñarro J, Aguilar MA (2013b). Effects of risperidone on the acquisition and reinstatement of the conditioned place preference induced by MDMA. *Brain Res Bull* **98**:36–43.

Roger-Sánchez C, Rodríguez-Arias M, Miñarro J, Aguilar MA (2013c). Involvement of 5-hydroxytryptamine 5-HT3 serotonergic receptors in the acquisition and reinstatement of the conditioned place preference induced by MDMA. *Eur J Pharmacol* **714**:132–141.

Schenk S (2009). MDMA self-administration in laboratory animals: a summary of the literature and proposal for future research. *Neuropsychobiology* **60**:130–136.

Schenk S, Gittings D, Colussi-Mas J (2011). Dopaminergic mechanisms of reinstatement of MDMA-seeking behaviour in rats. *Br J Pharmacol* **162**:1770–1780.

Shearman E, Rossi S, Szasz B, Juranyi Z, Fallon S, Pomara N, *et al.* (2006). Changes in cerebral neurotransmitters and metabolites induced by acute donepezil and memantine administrations: a microdialysis study. *Brain Res Bull* **69**:204–213.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, *et al.* (1999). Genetic enhancement of learning and memory in mice. *Nature* **401**:63–69.

Tomek SE, Lacrosse AL, Nemirovsky NE, Olive MF (2013). NMDA receptor modulators in the treatment of drug addiction. *Pharmaceuticals (Basel)* **6**:251–268.

Vidal-Infer A, Roger-Sánchez C, Daza-Losada M, Aguilar MA, Miñarro J, Rodríguez-Arias M (2012). Role of the dopaminergic system in the acquisition, expression and reinstatement of MDMA-induced conditioned place preference in adolescent mice. *PLoS One* **7**:e43107.